IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln No. : 10/600,266

Applicant(s): Fumitoshi ASAI et al.

Filed : June 20, 2003

For : MEDICINAL COMPOSITIONS CONTAINING ASPIRIN

Art Unit : 1614

Examiner : Brian Yong S. Kown

Docket No. : 03337C/HG

Confirm. No.: 7488

Customer No.: 01933

SECOND DECLARATION UNDER 37 CFR 1.132

Atsuhiro Sugidachi, declare that I am a co-inventor of the invention described and claimed in the above-referenced application.

1. I graduated from the Faculty of Pharmaceutical Sciences, Tohoku University, Japan, in 1987. I received a Ph.D. from the Faculty of Pharmaceutical Sciences of Tohoku University in 1996. I entered into the employment of Sankyo Co., Ltd., Tokyo, Japan, in April, 1989 and am now a senior chief researcher in Pharmacology and Molecular Biology Research Laboratories of the said company. From March, 2000 through February, 2002, I studied at the School of Medicine, the University of Pennsylvania, Philadelphia, USA.

I am a member of the following scientific societies:

The Japanese Pharmacological Society,

The Japanese Society of Thrombosis and Hemostasis.

Representatives of the scientific reports recently written by my co-workers and me are as recited below.

(1) "Antiplatelet action of R-99224, an active metabolite of a novel thienopyridine-type G_i-linked P2T antagonist, CS-747."; Br. J. Pharmacol. 132, 47-54 (2001)

(2) "Pharmacological profiles of R-96544, the active form of a novel 5-HT_{2A}, receptor antagonist R-102444."; Eur. J. Pharmacol. 457, 107-114 (2002)

(3) "Effects of R-102444, an orally active 5-HT_{2A} receptor antagonist, in rat models of peripheral vascular disease."; Vascular Pharmacology 41, 7-13 (2004)

(4) "Stereoselective inhibition of human platelet aggregation by R-138727, the active metabolite of CS-747 (Prasugrel, LY640315), a novel $P2Y_{12}$ receptor inhibitor."; Thromb. Haemost. 94, 593-598 (2005)

The following testing was accomplished under my supervision and control.

Experimental

Ex vivo platelet aggregation was measured in platelet-rich plasma (PRP) from male Sprague-Dawley rats. 2-Acetoxy-5-(a-cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine (hereinafter referred as Compound A, 3 and 6 mg/ml),

clopidogrel (100 mg/ml), and aspirin (10 mg/ml) were each suspended in a 5% (w/v) solution of gum arabic. Each test compound in combination with or without aspirin was orally administered to non-fasted rats in a volume of 1 ml/kg. The rats were anesthetized with pentobarbital (40 mg/kg, *i.p.*) 0.5 hr after the oral dosing, and blood was collected using 3.8% (w/v) sodium citrate solution (1/9 of the blood, v/v) as an anticoagulant. The PRP was prepared by centrifuging the blood at 230xg for 15 min at room temperature. Platelet-poor plasma (PPP) was obtained by a subsequent centrifugation of the remaining blood at 2,000xg for 10 min at room temperature. Platelet counts in PRP were determined using a hematology analyzer and adjusted to $5x10^8$ platelets/ml by the addition of PPP. Platelet aggregation was measured with a platelet aggregometer. PRP (240 µl) was placed into a cuvette, and prewarmed for 1.5 min at 37°C, and then stimulated with 10 µl of collagen (final concentration of 20 µg/ml).

Platelet aggregation was recorded for 7 min, and maximum aggregation was evaluated. The results of the platelet aggregation measurement are shown in the attached. These are expressed as the mean ± S.E.M. Statistical comparisons were carried out by Dunnett's test with respect to the control and by 2-way ANOVA for the assessment of any synergistic effect. A value of $p<0.05$ was considered to be statistically significant.

Discussion

As evidenced by the results of the comparison testing, the use of Compound A with aspirin provides surprisingly superior results to Compound A alone or aspirin alone. However, the results of the use of clopidogrel with or without aspirin do not show the surprisingly superior effect.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: April 27, 2006

A. Sugidachi

Atsuhiro Sugidachi